EFFECT OF LIPOPHILICITY ON THE RATE OF DIFFUSION OF SENSITIZERS INTO CELLULAR TARGETS

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SYNOPSIS

In a liquid-flow rapid-mix study, three radio-sensitizers of almost similar electron-affinity but differing partition coefficients were selected. The diffusion rate shown by a rise in enchancement ratio (ER) is found to increase with increasing lipophilicity. Hence, another factor apart from electron-affinity which would influence the amount of sensitization in site/s which occur either in lipidrich membrane or after penetration of such membranes is lipophilicity.

INTRODUCTION

Many nitromidazole compounds have been shown to sensitize hypoxic cells differentially to the lethal effect of ionising radiation when present at the time of irradiation (1, 2, 3, 4). The degree of sensitization by these compounds is found to be a function of the one electron-reduction potential, E of the compound. The higher the value of E, the smaller the amount of compound required to achieve a given level of sensitization (3, 4).

A successful model in drug design relates the concentration required to achieve a certain biological effect, C, with both the electronic term, E and the lipid: water partition term, P (partition coefficient) (5). This is given by:

$$-\log C = b_0 + b_1E + b_2 \log P + b_3 (\log P)^2$$

This model was applied to radiosensitization *in vitro* by a series of nitromidazole compounds which varied widely in E and P using mammalian cells (4). The authors concluded that P had little effect on C compared to E. In contrast, Anderson and Patel (6) observed a parabolic dependence of radiosensitising efficiency of nitromidazole compounds of similar electron-affinity on lipophilicity in *bacteria*. Using the above compounds of differing lipophilicity, Anderson and co-workers (7) proposed that one target of radiation damaged was the plasma membrane in *S. lactic* bacteria.

In vivo, a severe limitation to drug level and hence the degree of sensitization is its neurotoxicity (8, 9). Brown and Workman (10) tested in animal tumours ten 2-nitromidazole compounds of similar electron-affinity but differing partition coefficients. They reported that in normal brain, the concentration of drug decreased with decreasing lipophilicity. This would then reduce the degree of occurrence of central neuropathy and possibly also peripheral neuropathy. However the tumour/plasma ratio was found to be independent of the partition coefficient.

In the present study, three compounds of almost similar electron-affinity but differing partition coefficients were selected to study the time-resolved radiosensitization in Chinese hamster cells. A liquid flow rapid-mix apparatus was used in the experiments.

MATERIALS AND METHOD

Cells

Chinese hamster V79-379A cells were grown in spinner cultures in Eagles Minimal Essential Medium modified for suspension cultures supplemented with 7.5% foetal calf serum. Cells were kept in an exponential phase at concentrations between 10^5 and 10^6 cells per ml.

Rapid-Mix Apparatus

The apparatus used is as described previously (11). The experiments were performed under anoxic conditions.

Compounds

The radiosensitizers selected are shown in Table I. The three compounds had constant one-electron-reduction potentials (E_7^1s) within the experimental error of measurements.

Table I: The compounds selected for the experimental study.

Compound	E_7^1 (mV)	Partition Coefficient
Desmethylmisonidazole	– 389	0.11
Misonidazole	– 389	0.43
Benznidazole	– 380	8.2

RESULTS AND DISCUSSION

The survival curves of Chinese hamster, cells pre-irradiated (i.e. mixed prior irradiation) at some selected times for 50 mM desmethylmisonidazole and 0.5 mM benznidazole are shown in figures 1a and 2 respectively. Under pre-irradiation conditions, the rates of increase in ER were found to be different for the three selected compounds (figure 3).

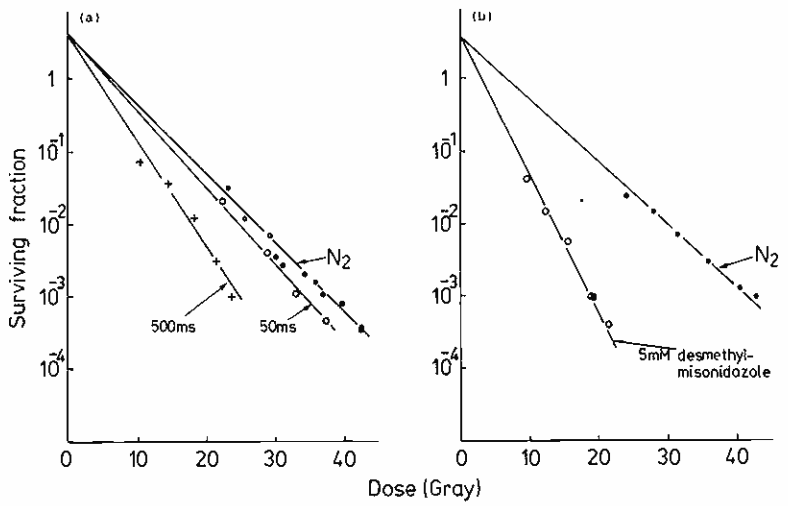

Figure 1: (a) Sensitization by 50 mM desmethylmisonidazole at pre-irradiation contact times of 50 ms (0) and 500 ms (+).
(b) Sensitization by 5 mM desmethylmisonidazole under stationary state conditions.

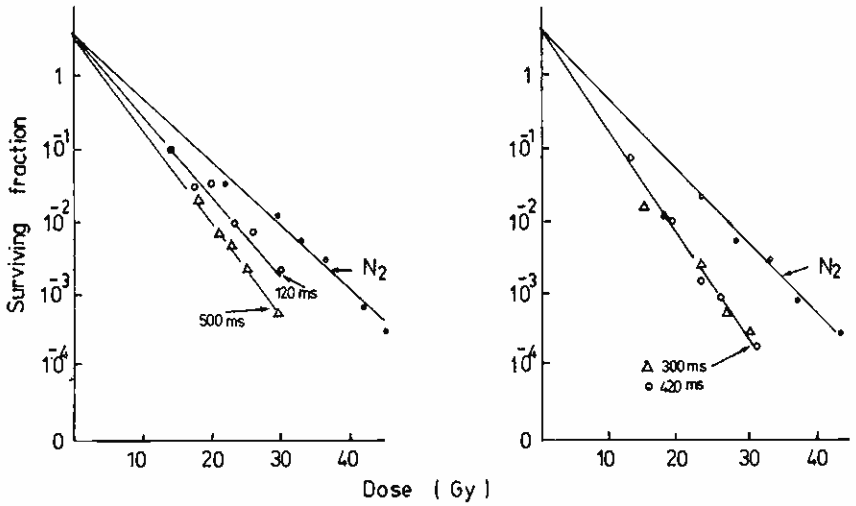

Figure 2: Effect of varying pre-irradiation contact times on sensitization by 0.5 mM benznidazole.

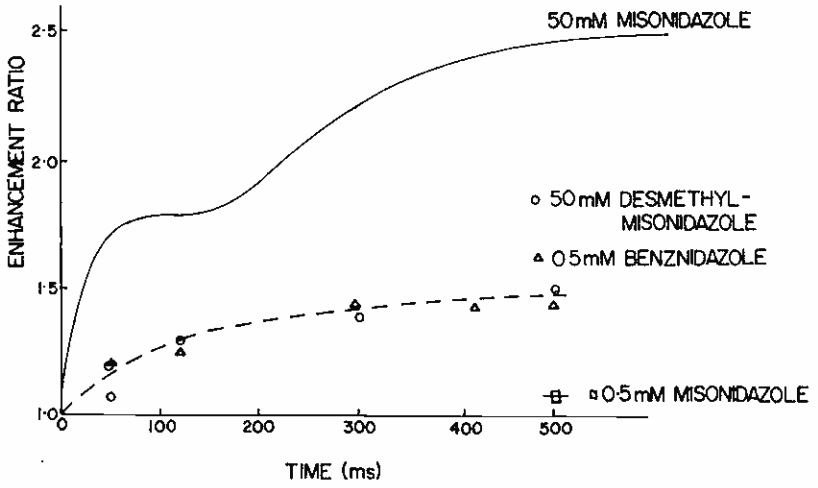

Figure 3: Dependence of enhancement ratio on pre-irradiation contact time for the given compounds at a known concentration. 50 mM misonidazole data (–) is taken from Kandaiya et al., 1980.

50 mM desmethylmisonidazole did not attain its maximum sensitization efficiency by 500 ms on the rapid-mix apparatus. it gave an ER of only 1.5 compared to an ER of 2.2 for 5 mM desmethylmisonidazole (figure 1b) under stationary-state conditions. In contrast, maximum sensitization was achieved by 50 mM misonidazole within 500 ms (11). On a 50 mM concentration basis, the rate of diffusion by desmethylmisonidazole into the cells is slower than that of misonidazole.

Benznidazole, at 0.5 mM concentration, gave an ER of 1.50 compared to an ER of 1.08 obtained with 0.5 mM misonidazole at 500 ms contact time. This indicates that benznidazole diffuses faster than misonidazole into the cells. Under stationary state conditions, an approximately similar ER of 1.8—1.9 was obtained for both 0.5 mM benznidazole and for 0.5 mM misonidazole.

For all the three compounds at the maximum concentrations used, the radiation-induced lesion is short-lived, since an ER 1.1 was obtained at 5 ms irradiation contact time prior mixing.

Since the three compounds are similar in electron-affinity, it is unlikely that the difference in the time scales of sensitization reflect differences in reaction rates. The more probable explanation is that the different rates of increase in ER reflect different rates of diffusion to the critical site/s within the cell. On this basis, the data for the three compounds show that the diffusion rate increases with increasing lipophilicity which agrees with the data of Anderson et al (7) in bacteria and that of Watts et al. (12) in Chinese hamster cells. In both the authors' experiments the pre-irradiation contact times were between 3 ms and 41 ms. The present experiment show that even at longer times i.e. from 5 ms to 500 ms, the full sensitization observed under stationary-state experiments is not obtained under rapid-mix conditions at low concentrations. These concentrations are normally administered in *in vivo* experiments. This, then strongly suggests that penetration of lipid-rich membranes is a critical factor in determining the overall rate of intracellular diffusion.

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